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Impact of Electron Beam Irradiation Treatment on Jujube for Fruit Flies (Diptera: Tephritidae) Disinfestation and its Impact on Quality Parameters

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ABSTRACT

This study examined the effects of electron beam irradiation on jujube infested with *Bactrocera zonata* (Saunders) and *B. dorsalis* (Hendel). Infested fruits were treated with five different doses of electron beam irradiation (100, 200, 300, 400, and 500 Gy) and stored at 16 ± 0.1°C with 85-90% relative humidity. The number of recovered pupae and emergence of adults from treated fruits were recorded. Physicochemical properties, fruit firmness, total soluble solid content, weight loss, and external fruit color were observed during the storage period. The findings showed that 200 Gy and above doses of electron beam irradiation significantly decreased pupal recovery and adult emergence from infested fruits. Furthermore, direct exposure of *B. zonata* and *B. dorsalis* larvae to 300 Gy resulted in mortality rates of 100% and 96.66%, respectively. Additionally, e-beam irradiation also enhanced fruit firmness, with 200 Gy doses indicating the best results. Moreover, the lowest total soluble solids were recorded in irradiated fruits compared with unirradiated fruits. The percentage of weight loss decreased significantly in all treatments, with 300 Gy demonstrating the lowest weight loss. Irradiation significantly enhanced the L* and b* (lightness and yellowness) of the external color of the fruits, while reducing a* (redness). This study demonstrates that electron beam irradiation is an effective method for controlling fruit fly infestation and maintaining the quality of jujube fruits during storage.

INTRODUCTION

The jujube (*Ziziphus jujuba* Mill.) is a plant belonging to the *Ziziphus* genus and the Rhamnaceae family. It is native to China and has been cultivated for over 4,000 years (Song *et al*., 2018). Jujube fruits are highly valued; however, they have a short shelf-life post-harvest (Cao *et al*., 2013; Tian *et al*., 2005). Consequently, it is advantageous for jujube producers to prioritize the cultivation of fruits with desirable characteristics, including firmness, vibrant color, uniformity, and elevated levels of ascorbic acid, total phenolic content, and total antioxidant activity during harvest and marketing. Such initiatives could enhance yields for jujube producers. According to the Government of Pakistan, the area dedicated to jujube cultivation in Pakistan during the 2022-2023 period is 3,607 hectares. Among the provinces, Sindh has the largest area under jujube cultivation, with 3,906 hectares, while Punjab has 1,223 hectares. In terms of production, Sindh yielded 14,330 tons of jujube, whereas Punjab produced 8,792 tons, resulting in a total national production of 24,635 tons (GoP, 2023). Known as the fruit of the poor, jujube is readily accessible and serves as a valuable source of nutrition. It contains carbohydrates, proteins, fats, vitamins, minerals, and alkaloids. Furthermore, fruit is rich in flavonoids and vitamins B1 and B2 and is recognized as a functional food due to its nutritional and medicinal properties (Huang *et al*., 2008).

Jujube fruit can be utilized to produce a diverse array of products, including pickles, jams, granulated sugar, confectionery, cakes, honey, sweets, soft drinks, marmalade, and both raw alcoholic beverages and liquor (Talpur *et al*., 2021). Furthermore, varieties of jujube fruit from China and India can be consumed fresh, dried, or

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processed into a variety of desserts, such as bread, cakes, compotes, and puddings (Krska and Mishra, 2008; Mishra and Krska, 2017).

Several jujube cultivars including Gola Early, Gola Lat, Gola Savina, Gola Curry (Black Gula), Gola Grape, Gola Grape, Sufi Local, White Chambeli, Mokhari, Goba, Ratam Chambeli, Nayab Chambeli, Sugat Golo, White Golo, Lumlat Golo, and Limay Golo, are grown in the Sindh province of Pakistan. While varieties, such as Karela, Kheri, Tukhmi, Omri, Dukil, Fuladi, Suvon, Anokhi, Mahmood Wali, Ajoba, Yazman Mahalli, Sadaqa, Pak White, Jorah, Jorah, seedless, Dalbaha, Delhi seed, Delhi seed, Bhawalpur selection 1, Bhawalpur selection 2, and Aloo Pokhara are grown in Punjab province of Pakistan (Talpur *et al*., 2021).

In jujube-growing countries worldwide, a total of 177 insect and non-insect pests have been identified as feeding on jujube. These pests are categorized as follows: Diptera - 13 species, Lepidoptera - 41 species, Coleoptera - 38 species, Hemiptera - 57 species, Hymenoptera - 1 species, Thysanoptera - 7 species, Isoptera - 3 species, Orthoptera - 2 species, Acariformes - 12 species, Gastropoda - 1 species, and Psittaciformes - 2 species. Among these, three species of fruit flies *Carpomyia vesuviana* Costa, *Bactrocera zonata*, and *Bactrocera dorsalis* are recognized as major pests of jujube. These species have been identified through Pest Risk Analysis (PRA) as having a high-risk potential (Balikai *et al*., 2013). Fruit flies can cause 90 to 100 % yield loss in fruits and vegetables depending upon several factors such as area season, variety and their population (Sapkota *et al*., 2010). Fruit flies caused direct loss in the form of yield and indirect loss such as reduction in trade and export prospect (Sharma *et al*., 2015).

Traditional control measures for fruit flies utilizing chemical insecticides present several disadvantages, including residual issues and the inability of these insecticides to penetrate infested fruits effectively to eliminate larvae. Furthermore, the growing public demand for insecticide-free fresh produce has prompted the adoption of environmentally friendly pest control methods (Dyck *et al*., 2005). Electron beam (e-beam) irradiation is a promising solution, approved by the USFDA (≤1 kGy) and IPPC (150 Gy for tephritids), offering advantages over gamma rays, such as no radioactive waste, higher dose rates, and compatibility with other treatments (Kong *et al*., 2014; Sandeep *et al*., 2023). E-beam disrupts pest DNA and microbial growth while delaying ripening by modulating cell wall enzymes (e.g., pectin methylesterase, polygalacturonase) and oxidative stress (Grasso *et al*., 2011; dos Passos Braga *et al*., 2020). Additionally, the International Plant Protection Convention has recommended a standardized dose of 150 Gy for tephritid fruit flies. Approved irradiation quarantine treatment doses for the melon fruit fly *B. cucurbitae* (Coquillet), Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), and Oriental fruit fly *B. dorsalis* (Hendel), which infest fruits and vegetables destined for export from Hawaii to the continental United States, are 210 Gy, 225 Gy, and 250 Gy, respectively (Yahia *et al*., 2019; Follett, 2024). E-beam irradiation is an effective postharvest fruit preservation strategy that has the potential to delay the onset of ripening and postharvest senescence, resulting in reduced fruit loss and extended shelf life (Yoon *et al*., 2020). When jujube fruits were irradiated at 0.25 kGy and 0.75 kGy, there was a significant reduction in ascorbic acid content at the end of the 30-day storage period at 12 °C. However, irradiation could be an effective approach to extend shelf life and maintain fruit quality when used in combination with hot water peeling and calcium chloride treatment (Sreejaya *et al*., 2012).

A study investigating ten jujube cultivars analyzed the organic acid content discovered that gamma radiation (0–5 kGy) significantly influences the color of the fruit and bioactive compounds, including phenols, anthocyanins, organic acids, and water-soluble vitamins. Specifically, irradiation up to 2.5 kGy slightly increased the total phenolic and monoanthocyanin content. These findings indicated that gamma irradiation can enhance jujube fruit quality at doses below 2.5 kGy (Najafabadi, 2017). Villavicencio *et al*. (2018) also concluded that e-beam irradiation is an effective method for food preservation. The recommended doses of this technique do not appear to adversely affect sensory quality, nutrient contents, or bioactive compounds in food. Compared to cobalt-60 irradiation, e-beam irradiation has several advantages, such as easy handling of the irradiated product and minimal spoilage of the irradiated product (Watanabe, 2000). Phytosanitary irradiation is currently approved and applied on a commercial scale. An e-beam is approved to meet quarantine requirements for exporting fresh fruits to markets with strict access requirements (Nguyen *et al*., 2022). Consequently, this study investigates the impact of e-beam radiation on the disinfestation of jujube fruit from *B. zonata* and *B. dorsalis* and the quality attributes, during the storage period. Additionally, the implementation of e-beam irradiation in Pakistan holds the potential to facilitate large-scale exports of jujube and other fruits, ultimately contributing to the country's foreign exchange earnings.

MATERIALS AND METHODS

Rearing and multiplication of fruit flies in the laboratory

Infested jujube fruits were collected from orchards near Tandojam in the Hyderabad district and subsequently placed in the Postharvest Laboratory of the Department of Plant Protection at Sindh Agriculture University for the purpose of adult emergence. Following emergence, the adults of various species were distinguished based on their morphological characteristics (Prabhakar *et al*., 2012).

The adults of each species were reared in separate cages. They were provided with a mixture of protein hydrolysate and sucrose at a ratio of 3:1 (v:v) and placed in Petri dishes (El-Gendy, 2017). Cotton soaked in distilled water was also provided and changed daily to prevent pathogen development. Gravid females were given semi-mature jujube fruit to facilitate oviposition. Infested fruits were transferred to cages containing sawdust to facilitate pupation and culture multiplication.

Fruit sampling

The White Gola is the most popular variety of jujube fruit in Sindh, Pakistan, was obtained from the market in Hyderabad, Pakistan. Fresh jujube of uniform size and maturity, without wounds or blemishes, was selected.

Fruit-based infestation and irradiation

Two species of fruit flies, *B. zonata* and *B. dorsalis*, were used in this study. The fruits were placed in separate cages and 30 pairs of fruit flies were released from the laboratory culture to infest the fruits. After 24 h, the infested jujube fruits were transferred to labeled cardboard boxes with five layers (measuring 40 × 20 × 10 cm) and transported to the commercial irradiation facility with an Electron-Beam-Accelerator (10 MeV) at the Pak Electron Beam Irradiation (Pvt.) Ltd. Port Qasim, Karachi, for treatment. Five different doses of electron beam irradiation (100, 200, 300, 400 and 500 Gy) were applied to the infested fruits. Following treatment, the boxes were moved to the laboratory for further data collection. The treated jujubes were shifted to labeled boxes and sawdust was provided for pupation. The sawdust was sieved weekly to recover pupae. The number of recovered pupae and adult emergence from each treatment were recorded. For larval irradiation treatment, 10 larvae per replicate (three replicates per dose) were placed in plastic cups and exposed to the same E-beam doses (100–500 Gy). Following irradiation, the cups were kept under room temperature at Laboratory Department of Plant Protection, Sindh Agriculture University. Daily observations were conducted to assess larval mortality.

Procedures for measurement of physiochemical quality parameters

Several physicochemical quality parameters were assessed, including fruit firmness, total soluble solids (TSS), weight loss, and external color of the fruits under cold storage at 16 ± 0.1 °C, utilizing two Split Air Condition unit (Dawlance Elegance 30) and maintaining a relative humidity (RH) of 85 - 90% through an Ultrasonic humidifier system (Model: JIAJIAHONG). Environmental conditions were continuously monitored with calibrated digital sensors (HTC-Electronic Temperature Humidity Meter), accuracy ±0.1 °C for temperature, ±1.5% for RH), and data were recorded at 3-days intervals.

Fruit firmness

Firmness was measured using a 'Digital Fruit Sclerometer' (AGY-15, China). The penetrometer was applied by firmly holding the sample in one hand, gripping the fruit meter between the two fingers of the other hand, placing the needle (with a thickness of 7.9 mm) above the sample, and pushing with increasing force until the needle penetrated the sample up to the mark. A slow puncture of the needle is necessary for accurate determination. The values obtained were expressed in kilograms (kg).

Sugar concentration

The sugar concentration was examined using a Digital Brix Refractometer (SOONDA, China). A standardized sample was prepared by crushing the skinned jujube pulp into a crusher. The sample was then gently stirred, and small droplets were placed on the spectrum of the Brix meter to determine their value by reading the display meter (AOAC, 2006).

Physiological loss in weight (PLW) was calculated by recording the weight before and after storage, expressed as a percentage of weight lost during storage, according to the following formula (Singh *et al*., 2016):

WL (%) = Fresh weigh – Weight at storage interval / Fresh weight × 100

Color measurements

The color of the samples was measured at specified time intervals during storage using a colorimeter (WR-10QC, Schenzen, China). In this color representation system (L*, a*, and b*) values represent a uniform three-dimensional color space, where L* corresponds to a dark-bright scale, a* is negative for green and positive for red, and b* is negative for blue and positive for yellow. The colorimeter was calibrated by using a standard white plate under normal light conditions (Mami *et al*., 2014).

Data analysis

The experiment was performed using a completely randomized design (CRD). Six treatments (100, 200, 300, 400, 500 and 0 Gy) were used, and each treatment was replicated three times. The collected data were analyzed using Statistix 8.1 statistical software. Analysis of variance

(ANOVA) was used to detect mean values estimated considering a significance level at ($p < 0.05$), and the least significant difference (LSD) test was used for pairwise comparisons to measure the significance of differences among means. Two-way ANOVA was performed, in which two factors (dose × storage duration) were used simultaneously to determine the effect of treatments.

RESULTS

Effect of e-beam irradiation on fruit flies

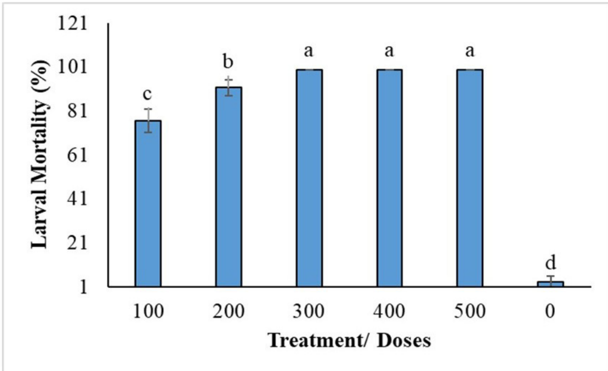
The findings presented in Table I indicate that when jujube fruits were treated with different doses of radiation (100, 200, 300, 400, and 500 Gy), the average number of *B. zonata* pupae collected were 36.33±3.25, 0.66±0.28, 0±0, 0±0, and(0±0, respectively. In contrast, the average number of pupae obtained from untreated jujube fruit was 377.33±41.23. A statistically significant difference was observed among all treatments ($F = 60.5$, $p = 0.0000$). A similar pattern was observed in jujube fruits infested with *B. dorsalis*. The highest average number of pupae was found in untreated fruits (343.33±25.16), followed by those treated with 100 (41.33±3.05), 200 (2.33±0.76), 300 (0±0), 400 (0±0), and 500 Gy (0±0). Additionally, no adult insects emerged from the treated jujube fruits. Untreated jujube fruits exhibited 100 percent emergence of *B. zonata* and 98 percent emergence of *B. dorsalis*.

Table I. Effect of E-beam irradiation treatment on *Bactrocera zonata and Bactrocera dorsalis* disinfestation on jujube fruit.

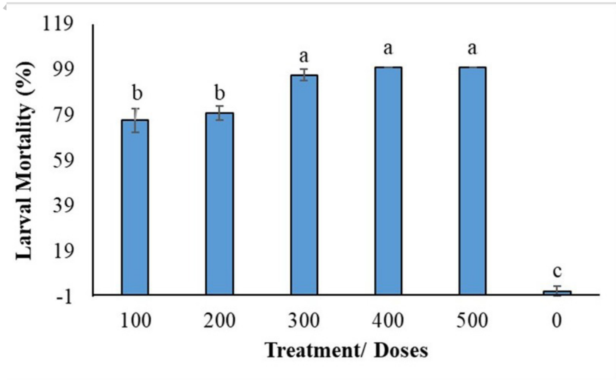
Doses (Gy)	*B. zonata*		*B. dorsalis*	
	Recovered pupae	Adult emer-gence%	Recovered pupae	Adult emer-gence %
100	36.33±3.25^{b}	0	41.33±3.05 ^{b}	0
200	0.66±0.28 ^{b}	0	2.33±0.76 ^{c}	0
300	0±0 ^{b}	0	0±0 ^{c}	0
400	0±0 ^{b}	0	0±0 ^{c}	0
500	0±0 ^{b}	0	0±0 ^{c}	0
0	377.33±41.23^{a}	100.00	343.33±25.16^{a}	98

Figure 1 showed that the irradiated larvae with higher doses above 300 Gy was observed (100%) larval mortality of *B. zonata* followed by 200 (91.66) and 100Gy (76.66%). However, the lowest larval mortality (3.33%) was observed in untreated larvae. Furthermore, results also indicated highly significant differences ($F= 299$, $p = 0.0000$) were recorded in irradiation doses and larval mortality.

The obtained results in Figure 1B indicate that the untreated larvae exhibited the lowest mortality of *B. dorsails* was recorded (1.67%). Whereas, increasing the radiation dose correspondingly elevated larval mortality rates, with 100% observed at higher doses of 400 and 500 Gy followed by (96.66%) at 300, (80%) 200 and (76.66%) at 100 Gy. The ANOVA analysis revealed a highly significant difference ($F= 248$, $p= 0.0000$) between the various irradiation doses and larval mortality.



A



B

Fig. 1. Effect of e-beam irradiation on larval mortality of *B. zonata* (A) and *B. dorsalis* (B).

Firmness

The effect of e-beam irradiation on jujube firmness is shown in (Fig. 3). The results evaluated that maximum firmness was found (13.11 to 7.07) at 200 Gy from 0 to the last day of storage followed by 300 (12.13 to 6.62), 100 (10.5 to 5.80) and 400 Gy (11.09 to 5.42). The minimum firmness was recorded (10.09 to 2.87) in untreated samples from day 0 to the last day of storage. Thus, the results also showed that a highly significant difference ($F= 121.13$, $p = 0.0000$) was recorded between the firmness of irradiated fruits at various storage times.

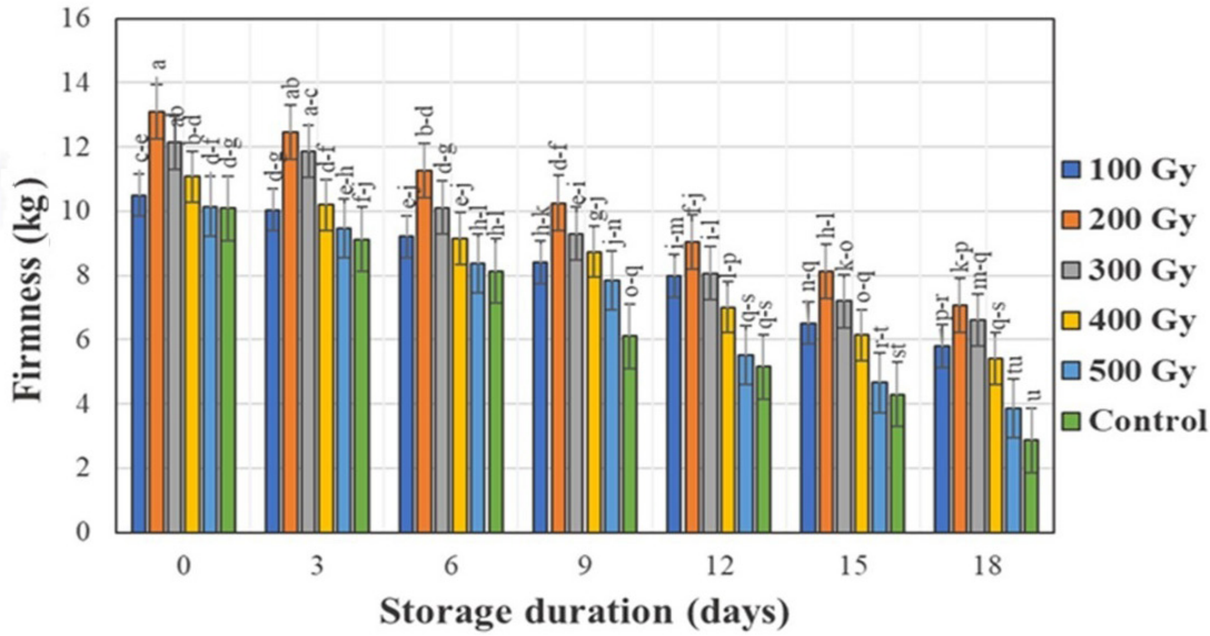


Fig. 2. Effect of e-beam irradiation treatment on firmness (Kg) of jujube fruit.

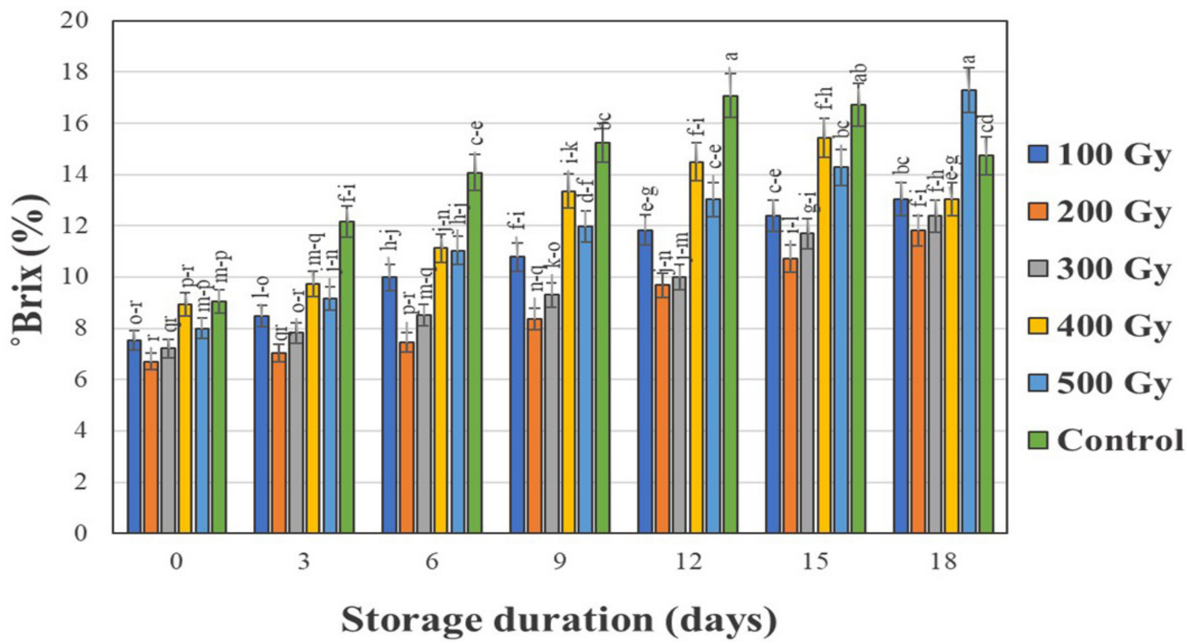


Fig. 3. Effect of e-beam irradiation on total soluble solid (°Brix %) of jujube fruit.

Total soluble solid (TSS)

The effect of e-beam irradiation on the TSS of jujube is shown in Figure 3. The results indicated that a highly significant difference ($F = 87.62$, $p < 0.05$) was recorded between various irradiation doses and the TSS of jujube. Thus, the results also showed that a highly significant difference ($F= 96.91$, $p < 0.05$) was recorded in irradiation doses and storage times. However, the maximum TSS was observed (17.30 °Brix) in untreated fruits after the 12 days of storage then gradually decreased until the last day. The

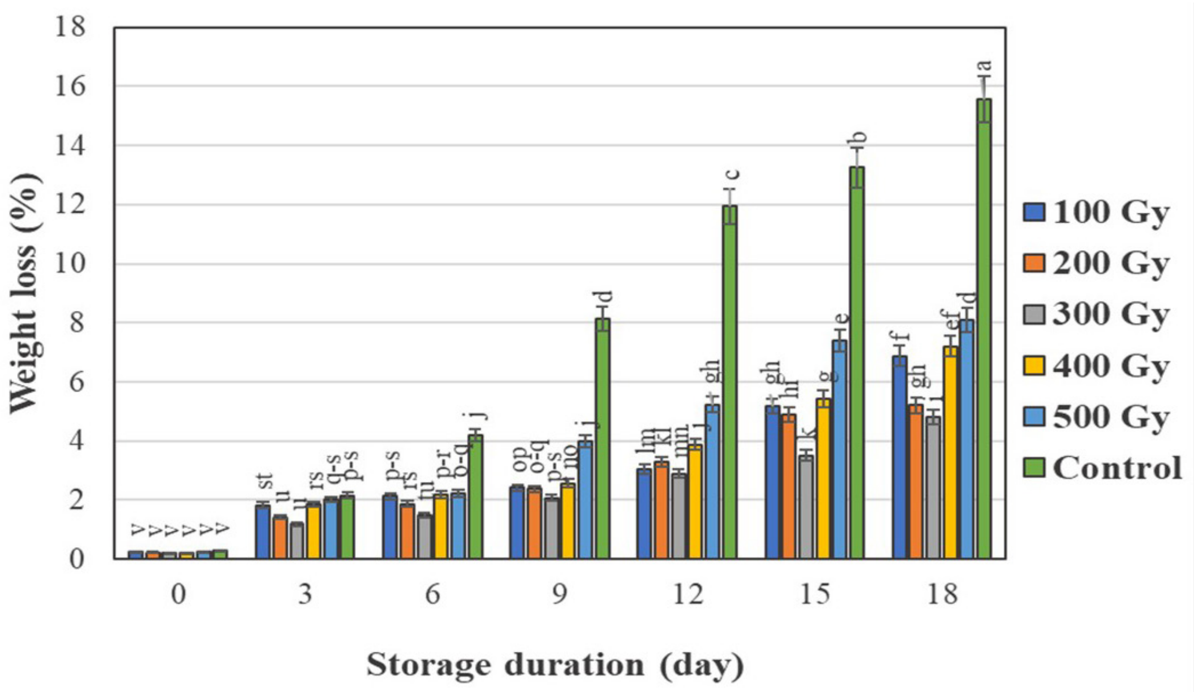


Fig. 4. Effect of e-beam irradiation on weight loss of jujube fruit.

lowest TSS (6.71 to 11.81 °Brix) was recorded at 200 Gy, followed by 300, (7.22 to 12.38°Brix) 100 Gy and 100 Gy (7.52 to 13.04°Brix) during 0 to 18 days storage period.

Weight loss

The results indicated that the weight of jujube fruits was significantly reduced in all treatments during storage. However, the highest weight loss was recorded (0.29 to 15.56%) during the first to last days of storage on untreated fruits. Whereas the lowest weight loss was recorded at 300 Gy (0.21 to 4.81%), followed by 200 Gy (0.23 to 5.21%), 100 (0.26 to 6.87%) and 400 Gy (0.22 to 7.21%). Furthermore, the results also showed a highly significant difference ($F = 1863.62$, $p = 0.0000$) among all treatments, weight loss, and various storage times (Fig. 4).

Fruit color

Figure 5A demonstrates the effect of e-beam irradiation on the color parameter L* (lightness). The results indicated that all the irradiation treatment doses significantly increased the L* (lightness) of the externally colored jujube fruits as compared to the control. The maximum L* value (54.15 to 59.41) was recorded on 300 Gy dose followed by 200 (53.05 to 58.23), 100 (50.11 to 55.01), 400 (45.14 to 51.82) and 500 (43.84 to 48.40) Gy at 0 to 18 day of storage periods. The minimum L* (35.27 to 43.89) was recorded for untreated fruits during storage. The results also indicated that a highly significant difference ($F = 187.31$, $P = 0.0000$) in the L* value was recorded at various irradiation doses. Thus, the results also showed that a highly significant difference ($F = 12.97$, $p = 0.0000$) was recorded between irradiation treatment and storage time.

The results also indicate that the color parameter a* represents red (+) to green (-), with more negative values indicating a greener color. This indicates a shift towards a greener color in jujube over time. The minimum a* value was recorded at 300 Gy (0.26 to 5.43), followed by 200 (1.01 to 6.41), 100 (1.35 to 6.79), and 400 (1.38 to 7.56) Gy during storage. However, the maximum a* value was recorded 1.75 to 11.43 in untreated fruits during 0 to 18 days of storage. Moreover, a significant difference was observed between the various treatments and storage durations (Fig. 5B). Additionally, another color parameter, b* (yellowness), was also examined (Fig. 5C), which significantly enhanced the b* value compared to the control during storage. The maximum b* value was recorded (19.02 to 34.67) at 300Gy. However, the minimum b* value was recorded (15.48 to 19.42) in untreated fruits. Thus, the statistical results also showed a significantly difference ($F = 61.13$, $p < 0.05$) between b* and treatments.

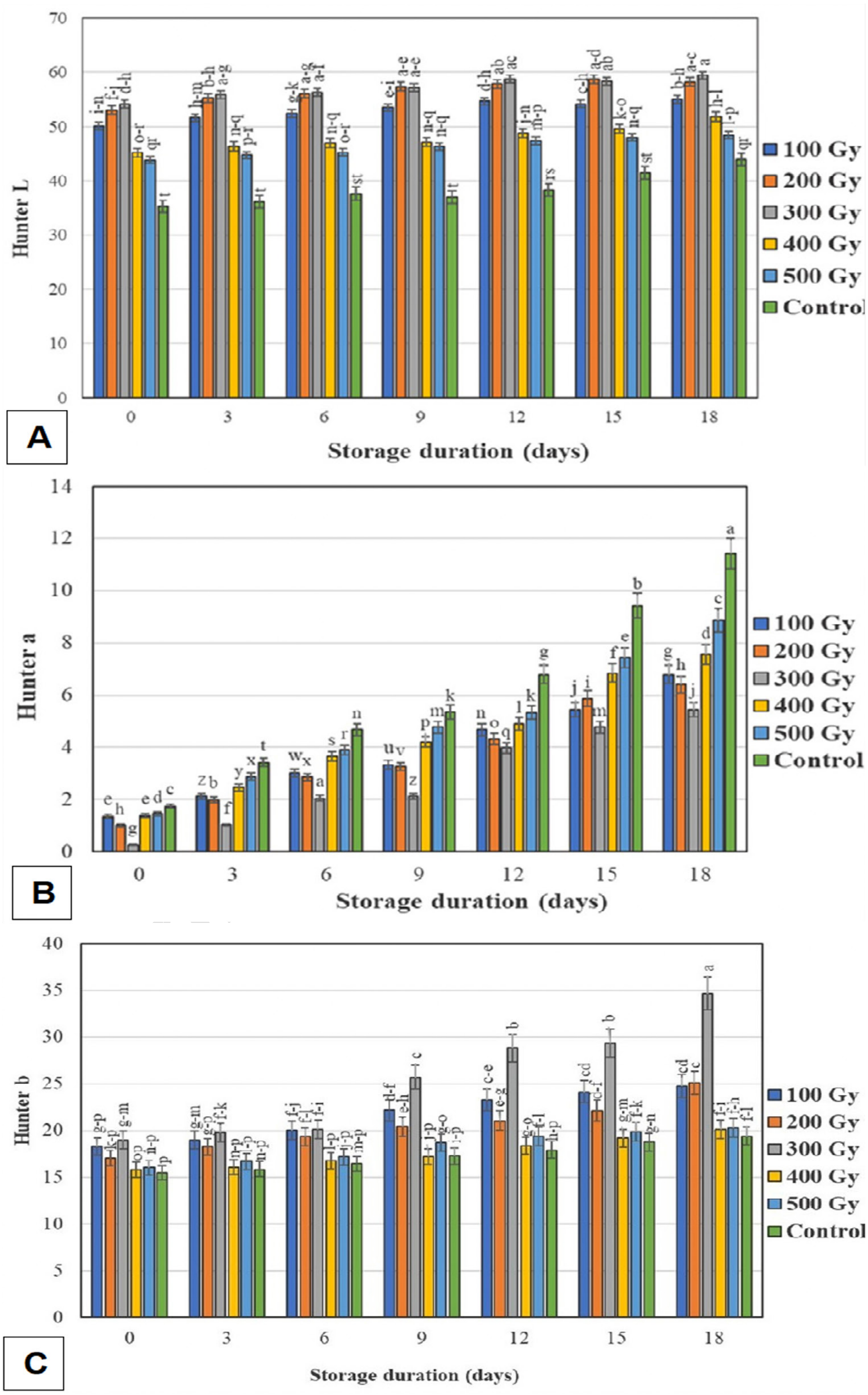


Fig. 5. Effect of e-beam irradiation on L parameters L*=lightness (A) (white L=100 to black L=0), “a” parameter (Redness) (B), and “b” parameter Yellowness (C) of jujube fruit.

DISCUSSION

Ziziphus jujube, part of the Rhamnaceae family, is cultivated in the hot regions of Sindh and Punjab, Pakistan. This fruit is not only flavorful but also utilized in canning, cooking, and confectionery. Jujube is a source of vitamins A and B, calcium, protein, fat, carbohydrates, and phosphorus. Various indigenous grafted jujube varieties have been developed in Sindh, and the area dedicated to jujube cultivation is expanding due to the fruit's export to Middle Eastern countries. Jujube is susceptible to a wide range of herbivorous insect pests, particularly fruit flies such as *C. vasoviana* Costa and *B. zonata*. Research by Mari *et al.* (2013) indicated that *Bactrocera* species caused damage ranging from 67% to 73% in different jujube cultivars in Pakistan. In contrast, Sachan (1984) reported that *Bactrocera* inflicted damage between 3.74% and 19.6% on jujube fruits. Damage levels can escalate significantly in the presence of a host fly infestation. Recognizing the importance of managing fruit flies, a study on electron beam treatment for the post-harvest disinfection of jujube fruit fly species has been conducted, as this method is considered safer for the ecosystem. Our findings demonstrated that higher doses of electron beam irradiation (greater than 300 Gy) effectively prevented the emergence of *B. zonata* and *B. dorsalis* pupae. Supporting our results, Draz (2016) reported a low incidence of pupae from treated fruits. Additionally, another study indicated that a dose of 116 Gy was adequate for disinfecting pupae, while a minimum dose of 100 Gy resulted in 100% pupal mortality (Zhao *et al.*, 2017). The mechanistic basis lies in DNA damage and reproductive sterility induced by ionizing radiation (Follett, 2024). Furthermore, previous research showed that a minimum dose of 95 Gy achieved complete sterilization (inability to reproduce) of *B. dorsalis* at seven days of age (Liang *et al.*, 2003).

The results of our study also showed that the maximum firmness and minimum weight loss of jujube fruits during storage were observed 200 and 300 Gy, respectively. Firmness retention is attributed to irradiation's suppression of pectin methylesterase (PME) and polygalacturonase (PG), which otherwise soften cell walls during ripening (Ayour *et al.*, 2021). Similar effects were observed in guava (Zhao *et al.*, 2017) and jujube (Guo *et al.*, 2022), where irradiation preserved texture without compromising soluble solids.

Color and TSS changes are linked to oxidative stress modulation. Irradiation reduces ROS accumulation, slowing chlorophyll degradation and sugar conversion (Hussain *et al.*, 2008; Grasso *et al.*, 2011). However, excessive doses (>2.5 kGy) may impair color development, as seen in mangoes (Thomas, 1986). Zhao *et al.* (2017) analyzed the nutrient content (sugar, sucrose, total sugar, titratable acid, vitamin C, and soluble solids concentrations) of irradiated and non-irradiated guava fruits and reported that no negative effects were observed on guava fruits at 600 Gy. Similar results have been reported in other studies that examined the effects of irradiation on the soluble solid content of other fruits (Shahbaz *et al.*, 2014). Furthermore, Guo *et al.* (2022) showed that irradiation with 0.5 kGy electromagnetic radiation prevented the decrease in firmness and increase in MDA content and delayed the increase in soluble solids content in winter jujube at the end of storage. Jat and Lakhawat, (2021) showed that electromagnetic radiation doses of 0.4 and 1.0 kGy effectively maintained the high firmness of winter jujube. E-beam's eco-friendliness, lack of residues, and compatibility with adjunct treatments (e.g., hot water, calcium chloride) make it viable for Pakistan's export goals. Economic feasibility hinges on scalability; while initial infrastructure costs are high, long-term benefits include reduced post-harvest losses (Rodrigues *et al.*, 2021) and access to regulated markets (Nguyen *et al.*, 2022). Future work should optimize dose-variety combinations and assess consumer acceptance.

CONCLUSION

In conclusion, the results of this study demonstrated that e-beam irradiation effectively disinfected both fruit flies, *B. zonata* and *B. dorsalis,* and free jujube fruits from infestation. All treatment doses significantly decrease pupal recovery and larval mortality and prevented adult emergence from treated fruits. Irradiation treatment also improved fruit firmness at a dose of 200 Gy. Moreover, the lowest total soluble solids were recorded in irradiated fruits compared with unirradiated fruits. The percentage of weight loss decreased significantly in all treatments, with 300 Gy demonstrating the lowest weight loss. Irradiation significantly enhanced the L* and b* (lightness and yellowness) of the external color of the fruits, while reducing a* (redness). This study demonstrates that electron beam irradiation is an effective method for controlling fruit fly infestation and maintaining the quality of jujube fruits during storage.

DECLARATIONS

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Generative AI and AI-assisted technology statement

The authors declare that they have not used generative

AI or AI-assisted technologies in this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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